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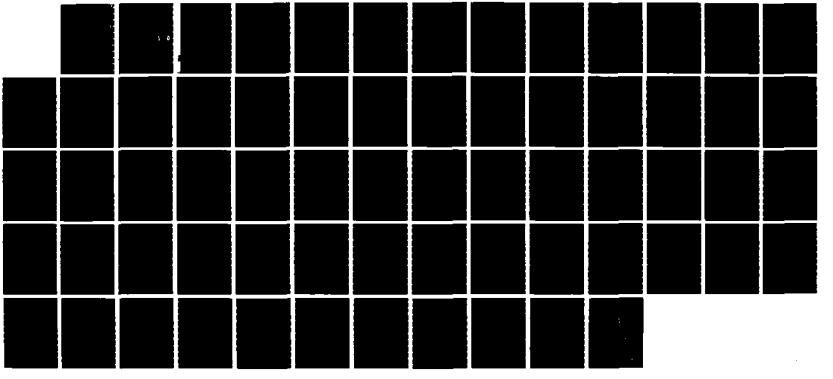

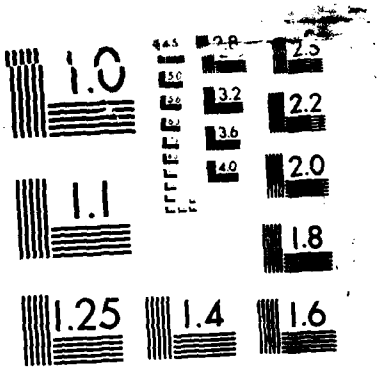
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RTI-31U-2597

SYNTHESIS OF NEW ANTIMALARIAL AGENTS

Annual Report

by

F. Ivy Carroll, Ph.D.
and
John A. Kepler, Ph.D.

May, 1985
(For the Period 1 April, 1984 to 31 March, 1985)

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-83-C-3099

Research Triangle Institute
Research Triangle Park, NC 27709

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Antimalarial Drug	Peroxides
Qinghaosu	Heterocycles
Primaquine	Synthesis

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This study is concerned with the preparation of: (a) peroxides based on qinghaosu, (b) peptide pro-drug derivatives of primaquine, and (c) thymidylate synthetase inhibitors as potentially new antimalarial agents. During the first year of this study, twelve (12) target compounds were prepared and submitted for antimalarial evaluation. An additional ten compounds were submitted during the second year of the project. The twenty-two compounds included sixteen (16) qinghaosu analogs, three (3) primaquine derivative and three (3) thymidylate synthetase inhibitors.

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Two peptide pro-drug derivatives of primaquine were tested for radical curative activity against Plasmodium cynomolgi in rhesus monkeys. When two monkeys were dosed at 1.0 mg/kg (this dose contains 0.319 mg/kg of primaquine) with the pro-drug D-Val-Leu-Lys-primaquine (RTI-2597-7), the monkeys relapsed on day 79 and day 65. Two monkeys have not relapsed after 30 and 31 days when dosed at 3.16 mg/kg (this dose contains 1.0 mg/kg of primaquine). Pro-drug D-Ala-Leu-Lys-primaquine (RTI-2497-14) did not cure one monkey at a dose of 1.0 mg/kg (this dose contains 0.319 mg/kg of primaquine). Two monkeys have not relapsed after 58 and 49 days when dosed at 3.16 mg/kg (this dose contains 1.0 mg/kg) of primaquine. These tests are still in progress.

Eight of the qinghaosu analogs and all three of the thymidylate synthetase inhibitors were evaluated in the malaria in vitro drug screen. The qinghaosu analogs showed reasonable activity in this screen, whereas the thymidylate synthetase inhibitors were inactive.

Sixteen target compounds were evaluated for blood schizonticidal suppressive activity against P. berghei in mice, and ten compounds were tested for causal prophylactic activity against P. berghei yoelii in mice. None of the compounds tested showed significant activity in these screens.

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Foreword

The research project entitled "Synthesis of New Antimalarial Agents" was the subject of an RTI renewal proposal dated August 30, 1983 to the U. S. Army Medical Research and Development Command. The renewal project was started April 1, 1984. H. A. Musallam of Walter Reed Army Institute of Research was the technical representative.

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1.0 Introduction

On August 30, 1983, we submitted a renewal proposal entitled "Synthesis of New Antimalarial Agents" to the U. S. Army Medical Research and Development Command. As a result of this submission, a contract (number DAMD17-83-C-3099) for the period April 1, 1984 to March 31, 1986 was awarded to the Research Triangle Institute (RTI). The contract states that RTI shall synthesize potential antimalarial compounds of the following types:

(a) Peroxides based on qinghaosu

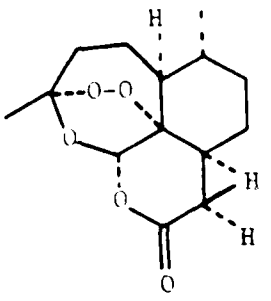
(b) Pro-drugs of primaquine

The following sections describe the work carried out on this contract during the period April 1, 1984 to March 31, 1985.

2.0 Synthesis

2.1 Analogs of Qinghaosu (1)

Chinese workers reported the isolation and characterization of a new schizonticidal agent, qinghaosu (1).[1,2] Qinghaosu is a sesquiterpene lactone



1

which contains the unique 5-oxygen substituted 1,2,4-trioxane ring system. Qinghaosu is of interest because of its low toxicity and its activity against chloroquine-resistant P. falciparum and cerebral malaria.[3]

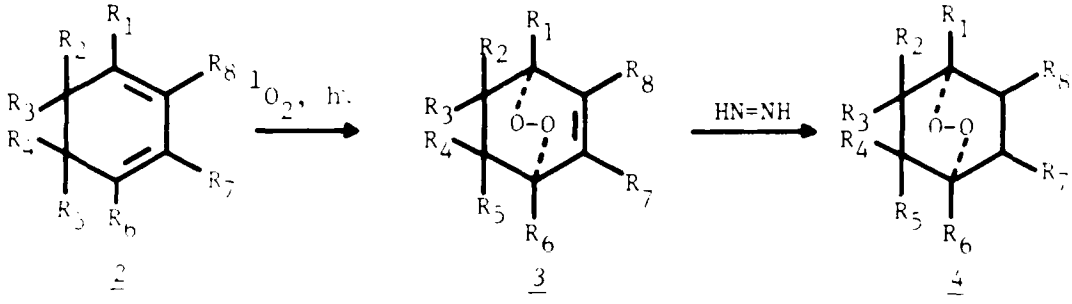
Studies have shown the peroxide linkage to be necessary for activity but nothing is known about the necessity of the remainder of the molecule. We believe the following points to be of particular interest: (a) is the peroxide linkage alone sufficient for activity, or is the complete 1,2,4-trioxane ring system required, (b) if the 1,2,4-trioxane ring system is also necessary how important is the 5-oxygen substituent. Answers to these questions may not only lead to a better antimalarial drug but also to a better understanding of the mechanism of action of qinghaosu.

The sensitivity of the peroxide function to most chemical reagents precludes doing structure modifications on qinghaosu itself, therefore we proposed to study the above points through model compounds. We proposed to prepare three types of compounds for testing; (a) cyclic peroxides, (b) 1,2,4-trioxanes, and (c) 1,2,4-trioxanes containing a 5-oxygen substituent. These

three sets of compounds separate the 5-oxa-1,2,4-trioxane ring of 1 into its component parts while maintaining the peroxide function. The following sections describe our progress during the last twelve months toward the synthesis of the types of compounds proposed.

2.1.1 Diimide Reduction of 2-Substituted-1,1,10-trimethyl-6,8-epidoxy-Δ^7-octalins

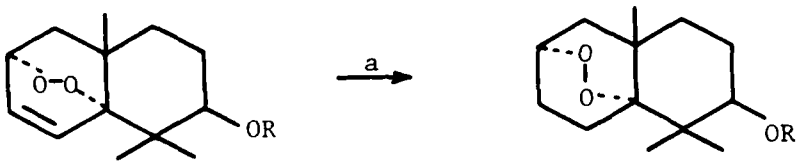
The general synthetic strategy in our preparation of cyclic peroxides (3) involves singlet photooxygenation of cis-diene (2) followed by selective reduction of the double bond to give 4 (cf. below). The singlet photo-



2 3 4

oxygenation reaction proceeded smoothly to afford the 6,8-epidioxy-Δ^7-decalins (5) (see Chart 1).[4] The reduction of double bonds in the presence of the endoperoxy function using diimide[5] generated from potassium azodicarboxylate (PADA) has been reported by Adam and Erden.[6] Previous work by Hamersma and Snyder[7] showed that the reproducibility of the diimide reduction is poor when comparing reductions with different batches of PADA or the same batch of PADA after storage for three weeks. We also experienced such inconsistent reactivity with different batches of PADA. We originally prepared PADA by Theile's[8] procedure, subsequently we generated PADA from commercially available azodicarboxamide. Our experience has indicated that the method of work up and purification of the PADA is important. The procedure reported by Berson et al.[9] in which the PADA is filtered, washed with methanol and dried

Chart 1



5 6

a) R = C_6H_5CO
b) R = C_4H_9OCO
c) R = CH_3CO
d) R = H

a) PADA, CH_3OH/AcOH

without recrystallization from aqueous alcohol has provided us with material of relatively consistent reactivity. It appears that the water content has direct bearing on the reactivity of PADA.[7]

In our initial attempts to reduce the epidioxy-Δ^7-decalins (5) to the corresponding saturated decalins 6 (Chart 1) with diimide we failed to achieve complete reduction. This was a serious problem because of the difficulty we encountered in separating 5 from 6. We found that complete reduction could be achieved if a 20-fold excess of PADA was used, and the reaction time was extended to 18 hr. Using these conditions we were able to convert 5a-d to their corresponding saturated compounds 6a-d in 74-87% yields.

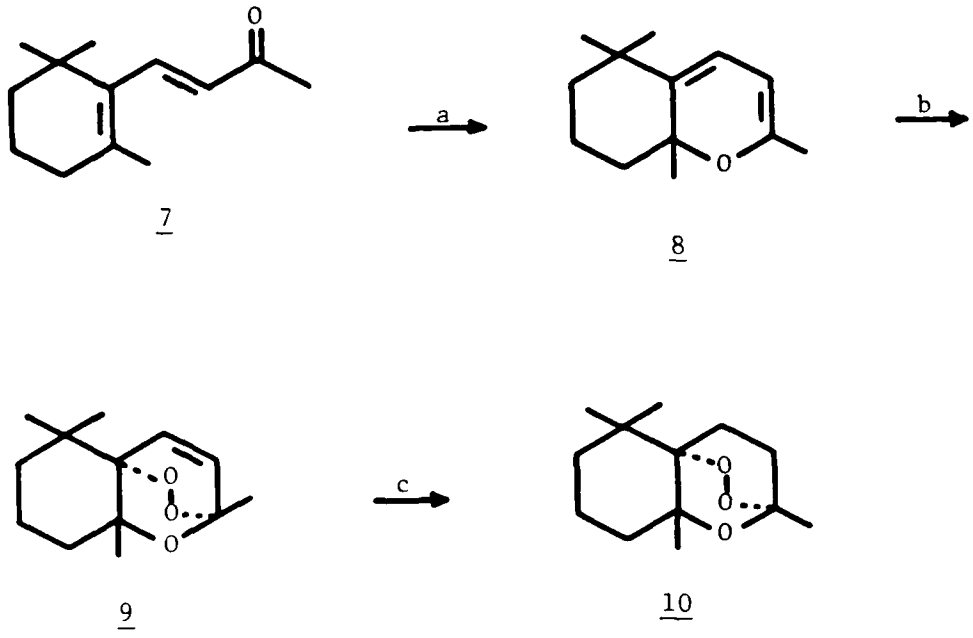
2.1.2 2,4a-Epidioxy-3,4,5,6,7,8-hexahydro-2,5,5,8a-tetramethyl-2H-1-benzopyran (10)

The title compound 10 was prepared by diimide reduction of $\Delta^{3,4}$-olefin (9)[4] as shown in Chart 2. The potassium azodicarboxylate (PADA) reduction of 9 gave excellent yields of the dihydro compound 10. Target compound 10 appears to be more stable than 9, probably because of reduced ring strain.

2.1.3 6,8a-Epidioxy-4a-methyl-2-oxo-3,4,4a,5,6,8a-hexahydro-2H-1-benzopyran (17a)

In order to test the efficacy of cyclic peroxides as antimalarial agents, we proposed to prepare the set of compounds 17 (Chart 3), where R can be H or CH_3. These compounds are of interest because they can be considered pro-drugs of the hydroperoxide group. The synthesis of the target compound 17a was accomplished by the sequence outlined in Chart 3. Cyanoethylation[10] of 2-methylcyclohexanone was effectively catalyzed by "Triton B" which is employed in the form of a methanolic solution (40% w/v) of trimethylbenzyl-ammonium hydroxide. Subsequent base hydrolysis gave 60% of the keto-acid 14a. Lactonization was effected by treatment with acetic anhydride and acetyl

Chart 2



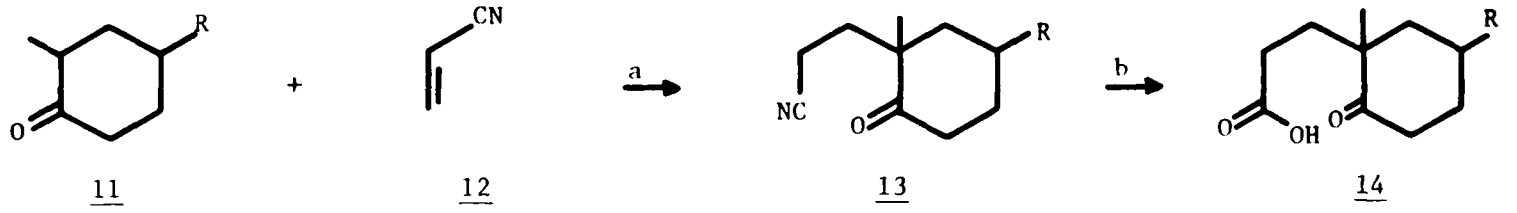
7 8 9 10

a) hν

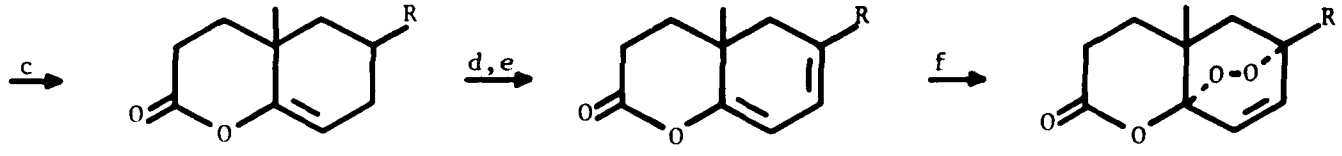
b) O_2, hν, Rose Bengal

c) PADA, AcOH-MeOH

Chart 3

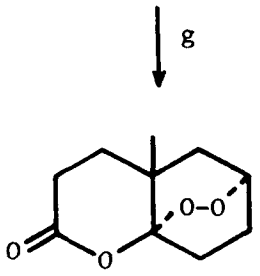


11 R = H or CH_3 12 13 14



15 16 17

a) R = H
b) R = CH_3



18

a) Triton B or $tBuO^-K^+$
b) NaOH
c) Ac_2O, AcCl
d) NBS, hν
e) N,N-Dimethylaniline, heat
f) $^1O_2$, hν, Rose Bengal
g) PADA, -20°

chloride.[11] The observation of a prominent IR band at 1680 cm^{-1} for the enolic double bond confirmed the presence of the enol lactone function. Allylic bromination and dehydrobromination proceeded smoothly to provide the diene-lactone 16a. Singlet photooxygenation of diene 16a in the presence of polymer bonded rose bengal dye gave the desired peroxide 17a.

2.1.4 4a,6-Dimethyl-6,8a-epidioxy-2-oxo-3,4,4a,5,6,8a-hexahydro-2H-1-benzopyran (17b)

The target compound 17b was prepared by the same scheme used to prepare its congener 17a. Our first attempts to carry out the cyanoethylation of 2,4-dimethylcyclohexanone (11b)[12] using Triton B as catalyst were not successful; however, a 57% yield of the desired product 13b was realized when the reaction was carried out using potassium tertiary butoxide as catalyst. The cyanoketone 13b was hydrolyzed to the keto-acid 14b in 98% yield. Treatment of 14b with acetic anhydride and acetyl chloride gave 70% of the enol lactone 15b. Allylic bromination-dehydrobromination of 15b afforded dienelactone 16b in 91% yield. Singlet photoxidation of 16b gave the endoperoxide target compound 17b in 43% yield.

2.1.5 6,8a-Epidioxy-4a-methyl-3,4,4a,5,6,7,8,8a-octahydro-2H-1-benzopyran (18a)

Earlier in this report (section 2.1.1) we described conditions for the successful diimide reduction of the Δ^7-octalin series. Attempts to apply these conditions to reducing the double bond of 17a was not successful. The major difficulties with the reaction was the formation of polar decomposition products and incomplete reduction. These problems were overcome by carrying the reaction out at -20°C rather than room temperature. Although 74% yield of material of 97% purity was obtained on a 100 mg scale, when the reaction was scaled up to 1.6 g, only 32% yield of product could be isolated. When 17b was

subjected to similar reduction conditions, the product, presumably 18b, showed a greater tendency to decompose than 18a. Because of this tendency to decompose, we have abandoned attempts to prepare 18b for the present.

2.1.6 Attempted Preparation of 2-Hydroxy Derivatives of 6,8a-Epidioxy-3,4,4a,5,6,8a-hexahydro-2H-1-benzopyrans

The synthesis of the 2-hydroxy derivatives of 6,8a-epidioxy-3,4,4a,5,6,8a-hexahydro-2H-benzopyran was proposed to extend the 2-oxo benzopyran series to a series that is somewhat more closely related to dihydroqinghaosu.

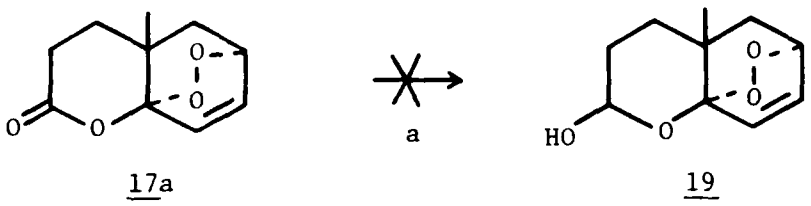
Attempts were made to reduce the epidioxylactone 17a and 18a to the corresponding lactols 19 and 20 using sodium borohydride under conditions used for the reduction of qinghaosu to dihydroqinghaosu,[1,13] but without success (Chart 4). Decomposition rather than reduction appeared to occur under the conditions employed.

An alternate method for preparing the desired compounds (Chart 5A) was also investigated. The key step in this scheme is the reduction of the enol-lactone function to the enol-lactol, i.e. 15a to 21. This conversion was attempted under various conditions without success. The primary products were unreduced starting material and overreduced product, keto-alcohol 26 (see Chart 5B). In one case the keto-aldehyde 25 was detected in small quantity by mass spectrometry and ^{1}H NMR (minor resonance at δ 9.62 for the CHO function). The detection of 25 and 26 in this reaction is discouraging because it implies that the desired enol-lactols 21 are not stable under the reduction conditions, and are more easily reduced than the enol-lactones either directly or through the corresponding keto-aldehyde as shown.

2.1.7 11,12,13-Trioxatricyclo[7.2.2.$0^{4,9}$]tridecane Analogs of Qinghaosu

Preliminary work on the synthesis of the trioxatricyclotridecane analogs of qinghaosu has been initiated. The proposed synthetic scheme for preparation

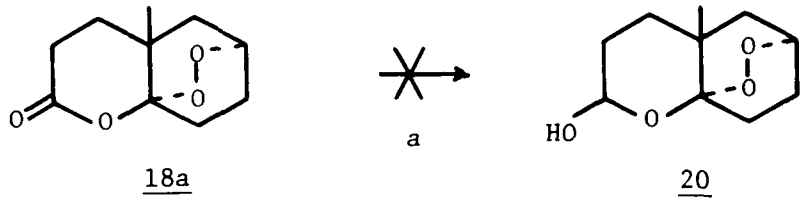
Chart 4



17a

a

19



18a

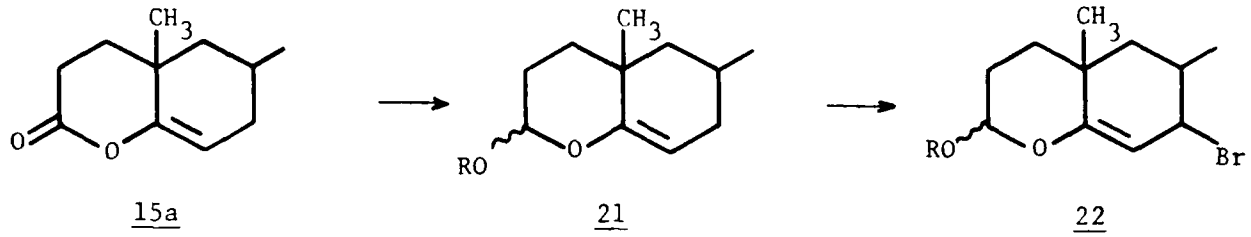
a

20

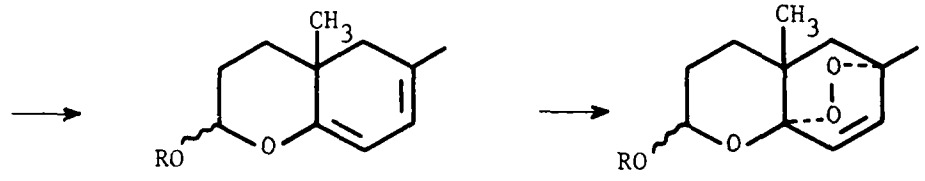
a) $NaBH_4$, MeOH

Chart 5

A



15a 21 22

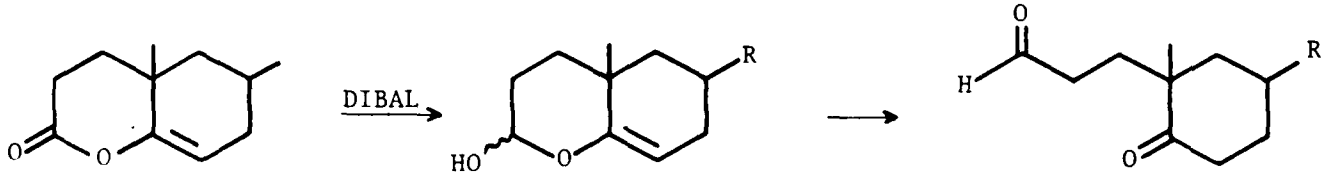


23 24

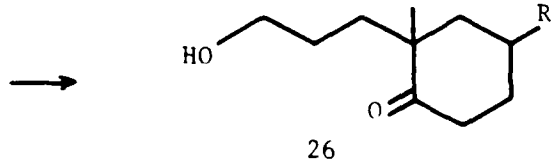
a) R = H

b) R = CH_3

B



15a 21a 25



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of this series is outlined in Chart 6. Conversion of the previously described enone 27[14] to dienone 29b was accomplished via chloranil in refluxing t-butanol.[14] Copper acetate catalyzed 1,6-addition of methyl magnesium bromide to 29b gave 58% of the desired 7,10-dimethyl-Δ^8-octalin 30b; however, an approximately 30% yield of the 1,2-addition product was formed as by-product. The use of dimethyl lithium cuprate in this reaction eliminated the side products, and gave 73% of the pure 1,6 adduct.[15] It has been shown that cuprates based on copper cyanide and two equivalents of organolithium give results superior to the dialkyl lithium cuprates.[16] Use of this "higher order" cuprate gave an 85% yield of 30b with the absence of side products. The excellent yields, combined with the ease of use and lower costs, make this the method of choice for performing the 1,6-addition reaction.

The unsaturated ketone 30b was immediately reduced via DiBAL at -78° to yield a mixture of products. The major component was assigned structure 31b on the basis of its ^{1}H NMR spectrum and its conversion to 32b.

Conversion of 31b to the acetate 32b was carried out using acetic anhydride catalyzed by triethylamine and dimethylaminopyridine[17] and was quantitative. This brings us within two steps of the target compound 34b.

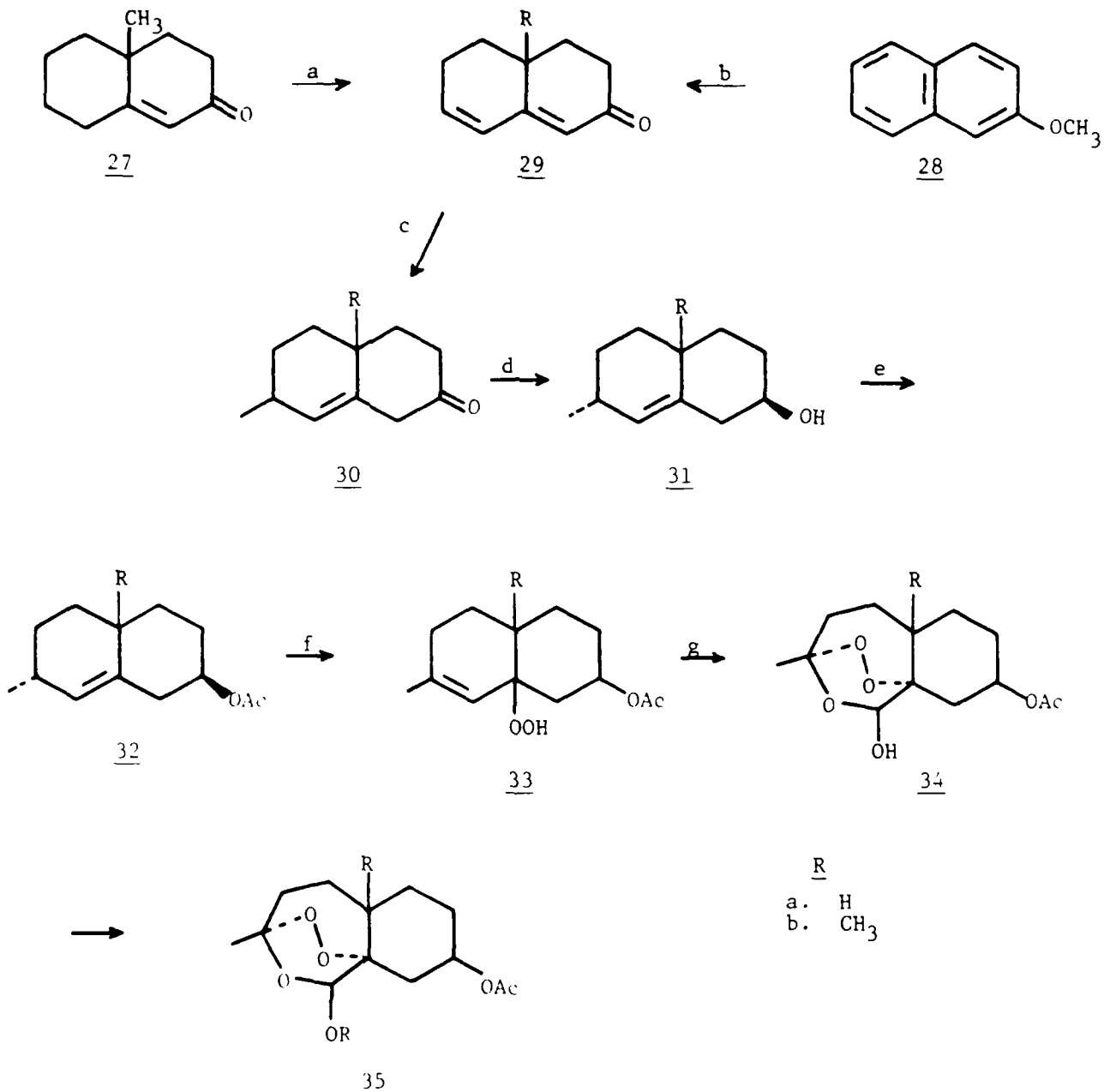
The above series of reactions has also been carried out on the dienone 29a. This unsaturated ketone arises from the Birch reduction of 28 followed by reflux in dilute acid.[18]

Treatment of 29a with the dimethyl dilithium cyano cuprate gave the unsaturated ketone 30a in 86% yield. Reduction of 30a with DIBAL at -78°C gave a mixture of which the major product was alcohol 31a. The acetate 32a was prepared as described for 32b.

2.1.8 8a,3-Epidioxy-3-methyl-1-oxo-5,6,7,8-hexahydro-1H-2-benzopyran

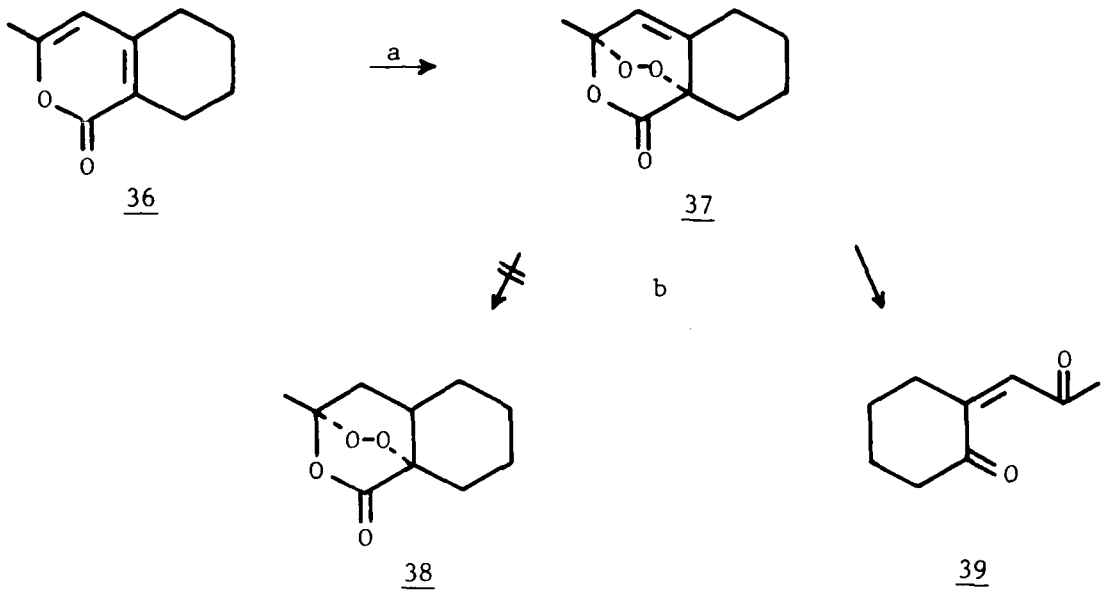
8a,3-Epidioxy-3-methyl-1-oxo-5,6,7,8-tetrahydro-1H-2-benzopyran (37) was prepared by singlet oxygen addition to the α-pyrone 36[4] (Chart 7).

Chart 6



a) Chloranil
b) Birch reduction, H_3^+O
c) $Me_2CuCNLi_2$
d) $NaBH_4$
e) Ac_2O, Pyr.
f) $^1C_2$, hν, sensitizer
g) O_3, CH_2Cl_2, Pyr.

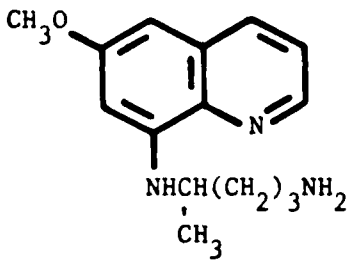
Chart 7



Unfortunately 37 was unstable and decomposed even at low temperature storage. Since we expected the dihydro product 38 to be more stable, we investigated the reduction of 37 to 38. However, several attempts to achieve the conversion of 37 to 38 resulted in oily decomposition products. The major decomposition product gave spectral data consistant with diketone 39, which is the product resulting from loss of carbon dioxide.

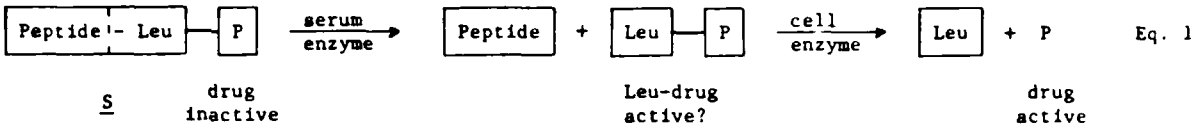
2.2 Peptide Derivatives of Primaquine

In a recent report Trouet and co-workers[19] reported the preparation, antimalarial activity, and toxicity of several amino acid and peptide derivatives of primaquine (P). The activity was assessed against P. berghei in mice by a procedure which was designed to show causal prophylactic antimalarial activity. The toxicity was established by determining the LD_{50}.



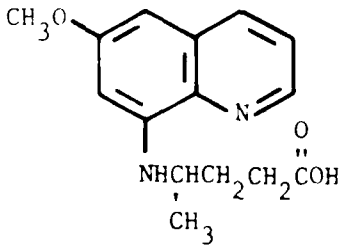
P

Trouet and co-workers[19] prepared Leu-P, Ala-Leu-P and Ala-Leu-Ala-Leu-P and found that Leu-P was not hydrolyzed in the presence of serum whereas Ala-Leu-P and probably Ala-Leu-Ala-Leu-P were slowly hydrolyzed to release Leu-P. Ala-Leu-P and Ala-Leu-Ala-Leu-P were found to be less toxic and more active than primaquine while Leu-P showed activity and toxicity similar to primaquine. The authors suggested that Ala-Leu-P and Ala-Leu-Ala-Leu-P are products which have to be hydrolyzed to Leu-P in the serum in order to be active. Since Leu-P is not hydrolyzed in serum, it is either active itself or hydrolyzed to primaquine inside the cells. Equation 1 summarizes this process.



P = primaquine

The reasons why the peptide derivatives of primaquine are less toxic and more active than primaquine (P) are not known. Trouet and co-workers[19] suggest that the uptake and distribution of the peptide pro-drugs in the tissue are different from those of P and thus might explain their different chemotherapeutic properties. Baker and co-workers[20] found that 35 to 83% of the primaquine dose was converted to 8-(3-carboxy-1-methylpropylamino)-6-methoxyquinoline (40) in Rhesus monkeys. Interestingly, the plasma concentration of 40 exceeded that of P within 15 minutes. The peptide residue may serve to protect the primary amino function of P against rapid metabolism.



40

Regardless of the mechanism of their operation, the extremely encouraging results of Trouet and co-workers[19] suggest the synthesis of other amino acid and peptide derivatives of P as a means of improving the chemotherapeutic properties of P. Chart 8 outlines the synthesis of four new peptide derivatives of primaquine (P) which have been designed to enzymatically liberate

free P in a manner to achieve selective toxicity. The synthesis of 4'-N-(D-Val-Leu-Lys)primaquine (46a) was described in our first annual report.[4] The synthesis of two other compounds are presented in the following sections.

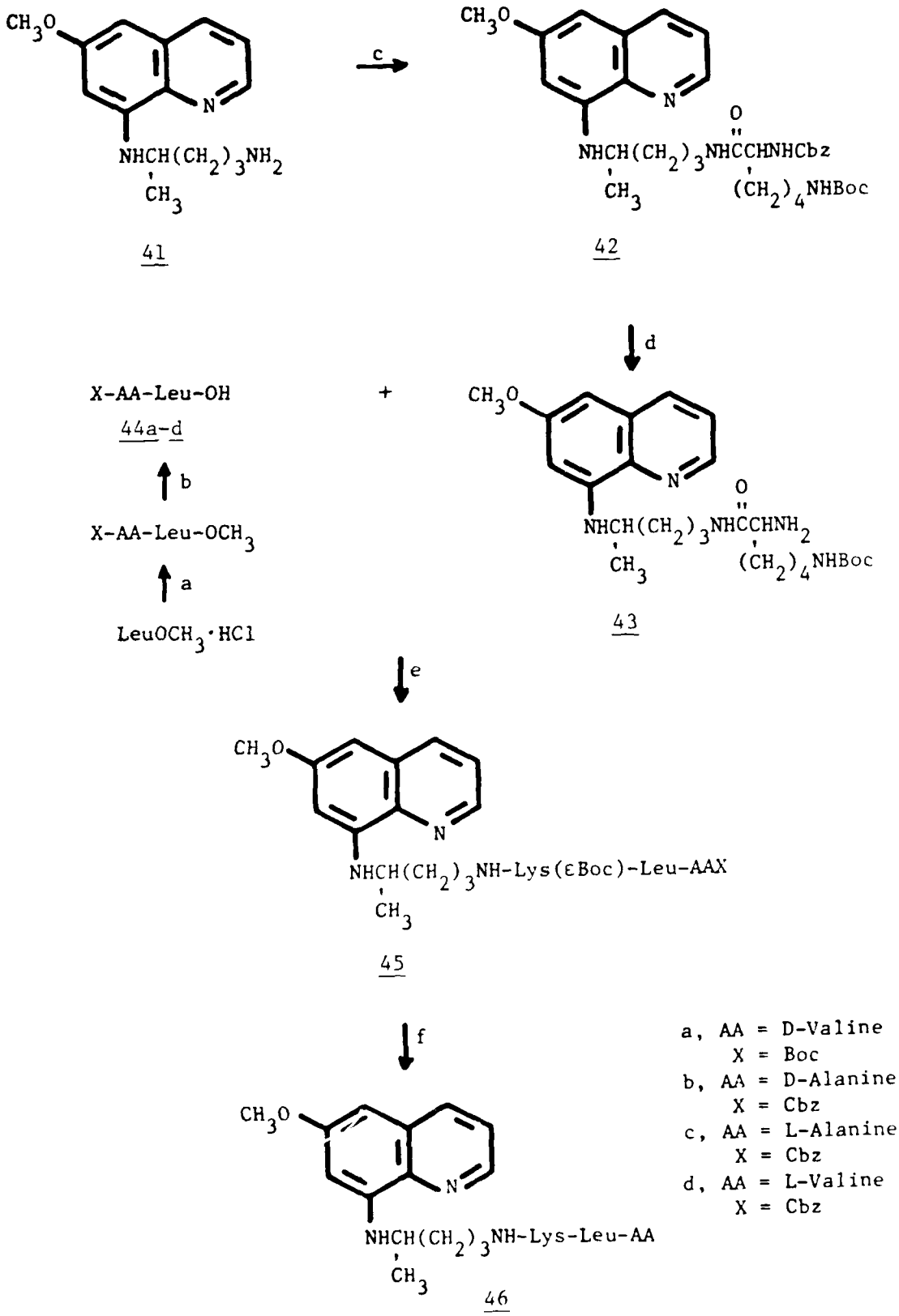
2.2.1 4'-N-(D-Ala-Leu-Lys)primaquine (46b)

The synthesis of 46b is shown in Chart 8. The free amino compound 43[4] was coupled with Cbz-D-Ala-Leu-OH (44b) to give 45b which was purified by column chromatography. The dipeptide (44b) was prepared from leucine methyl ester and Cbz-D-alanine using a procedure similar to that used for 44a.[4] The NMR spectrum of 44b showed the characteristic resonances at 0.92 and 1.38 ppm for the isopropyl methyl groups of leucine and the methyl group of alanine, respectively. Since the amino groups in tripeptide 45b were protected by the CBz group as well as the BOC group, a two step deprotection was needed. Removal of the BOC group proceeded smoothly using trifluoroacetic acid in methylene chloride. The completion of the reaction was confirmed by NMR spectroscopy as well as by TLC analysis. The resulting product was subjected to hydrogenolysis using 70% Pd/C catalyst to remove the CBz group. The crude residue obtained was purified by column chromatography (silica gel) and converted to the phosphate salt.

2.2.2 4'-N-(Val-Leu-Lys)primaquine (46d)

The dipeptide 44d was prepared by saponification of commercially available Cbz-Val-Leu-OCH_3 and was coupled to 43 using the DCC method to afford the diprotected peptide conjugate 45d. The protective groups were moved by hydrogenolysis, followed by treatment with trifluoroacetic acid to give the free base 46d. The free base was purified by column chromatography and converted to the phosphate salt.

Chart 8



a, AA = D-Valine
X = Boc
b, AA = D-Alanine
X = Cbz
c, AA = L-Alanine
X = Cbz
d, AA = L-Valine
X = Cbz

a) Et_3N, HOBT, Boc-D-Val, DCC b) NaOH, CH_3COCH_3, DMF c) Et_3N, HOBT, N^{ε}-Boc-NCbz-Lys, DCC
d) Pd/C, H_2 e) HOBT, DCC f) CF_3CO_2H, CH_2Cl_2

3.0 Biological Test Data

3.1 Malaria in Vitro Drug Screen

Eight of the sixteen qinghaosu analogs submitted for antimalarial evaluation were tested in the malaria *in vitro* drug screen. The data is summarized in Table 1. With the exception of RTI-2597-8 and RTI-2597-11, all of the qinghaosu analogs have shown activity in this screen. Target compound RTI-2597-12 has the lowest ED-50 in the Camp strain, whereas RTI-2597-9 and RTI-2597-10 possess the lowest ED-50 for the Smith strain.

3.2 Plasmodium Berghei Tests in Mice

Test results on *Plasmodium berghei* were carried out by the Rane Malaria Screening Laboratory, University of Miami, Miami, Florida.[21] The test results were supplied by Walter Reed Army Institute of Research, Washington, D. C. In the primary test against *Plasmodium berghei* five mice were infected with a lethal dose of *Plasmodium berghei* three days prior to administration of the chemical. Routinely the chemical was administered subcutaneously in sesame or peanut oil. The mean survival time (MST) of infected control mice is 6.2 $\pm$ 0.5 days. Extension in survival time (ΔMST) of the chemically treated mice is interpreted as evidence of antimalarial activity. Compounds are arbitrarily considered to be "active" when the mean survival time of the treated group is more than twice the mean survival time of the control group. Mice surviving 60 days are considered cured.

Test results from sixteen (16) compounds are summarized in Table 2. The only compound that showed activity in this screen was the primaquine pro-drug analog RTI-2597-7 which has a Lys-Leu-D-Val peptide connected to the terminal side chain amino function. This compound was active at 160, 320 and 640 mg/kg but was also toxic at 320 and 640 mg/kg. Surprisingly, the primaquine pro-drug analog RTI-2597-16 which has a Lys-Leu-Val peptide connected to the

Table 1. Malaria in Vitro Drug Screen Data

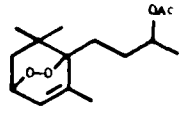
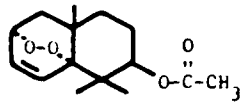
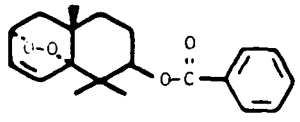
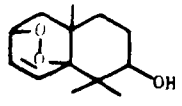
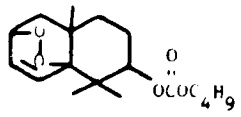
RTI No./ Structure	WR No./ Bottle No.	Malaria in Vitro Drug Screen (ng/mL)	
		Camp	Smith
RTI-2597-4		a	a
			
RTI-2597-5	251766 BK69892	ED-50 = 239.34 (202.76-275.91)	ED-50 = 164.00 (158.73-169.26)
			
RTI-2597-6	251765 BK69909	ED-50 = 340.00 (319.87-360.13)	ED-50 = 402.15 (390.42-413.87)
			
RTI-2597-8	251848 BK71132	a	ED-50 = INIT CONC TOO LOW
			
RTI-2597-9	251849 BK71141	ED-50 = 100.15 (96.48-103.82)	ED-50 = 57.19 (54.49-59.90)
			

Table 1 (continued)

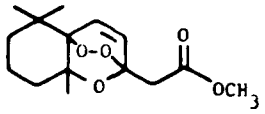
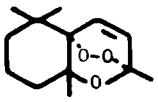
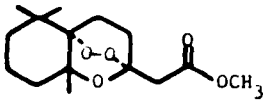
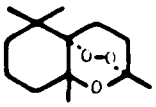
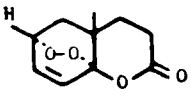
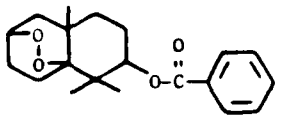
RTI No./ Structure	WR No./ Bottle No.	Malaria in Vitro Drug Screen (ng/mL) Camp	Smith
RTI-2597-10 	251847 BK71150	ED-50 = 95.69 (87.06-104.33)	ED-50 = 38.93 (37-57-40.30)
RTI-2597-11 	252015 BK73145	ED-50 = INIT CONC TOO LOW	ED-50 = INIT CONC TOO LOW
RTI-2597-12 	252067 BK73798	ED-50 = 83.24 (81.80-84.69)	ED-50 = 74.93 (73.64-76.23)
RTI-2597-13 	252128 BK74482	ED-50 = 1351.76 (1231.27-1472.25)	ED-50 = 1000.79 (660.05-1341.52)
RTI-2597-15 		a	a

Table 1 (continued)

RTI No./ Structure	WR No./ Bottle No.	Malaria *in Vitro* Drug Screen (ng/mL)	
		Camp	Smith
RTI-2597-17 		a	a

a) Data has not been received for these compounds.

Table 2. Antimalarial Test Results against P. Berghei

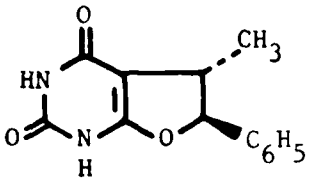
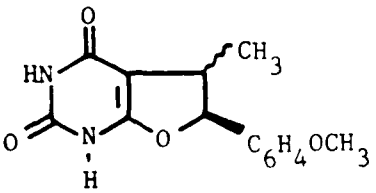
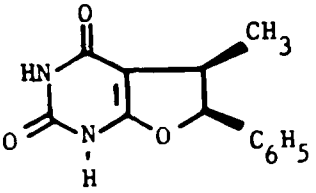
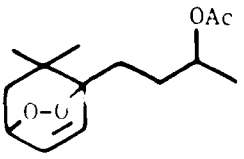
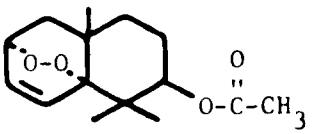
Identification[a]	Structure	Dose mg/kg	T-C[b]	Toxic Deaths[c]	MST[d]	Comments
RTI-2597-1 BK64995		40 160 640	0.5 0.1 0.5	0 0 0	0 0 0	
RTI-2597-2 BK69758		40 160 640	0.1 0.5 -0.1	0 0 1	0 0 3	Toxic
RTI-2597-3 BK64986		40 160 640	-0.1 -0.1 0.1	0 0 0	0 0 0	
RTI-2597-4 BK69641		20 80 320	-0.1 0.1 0.3	0 0 0	0 0 0	
RTI-2597-5 BK69892		40 160 640	-0.1 -0.1 0.3	0 0 0	0 0 0	

Table 2 (continued)

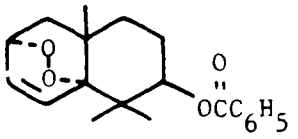
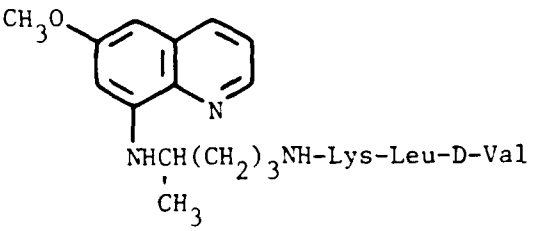
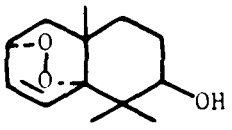
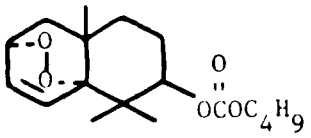
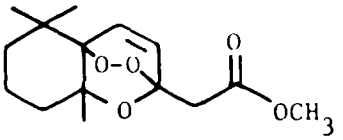
Identification[a]	Structure	Dose mg/kg	T-C[b]	Toxic Deaths[c]	MST[d]	Comments
RTI-2597-6 BK69909		40 160 640	0.7 0.1 0.3	0 0 0	0 0 0	
RTI-2597-7 BK70484		20 40 80 160 320 640	1.5 2.5 5.3 6.9 7.6 9.9	0 0 0 0 2 3	0 0 0 0 3 3	 Active Active Active
RTI-2597-8 BK71132		40 160 640	-0.1 0.1 0.4	0 0 1	0 0 3	 *Toxic*
RTI-2597-9 BK71141		40 160 640	-0.1 0.1 1.1	0 0 0	0 0 0	
RTI-2597-10 BK71150		40 160 640	-0.1 0.1 -	0 0 5	0 0 3	 Toxic

Table 2 (continued)

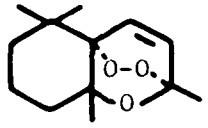
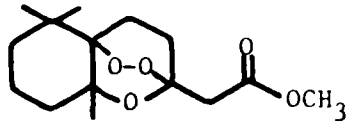
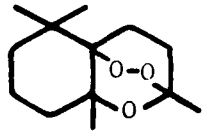
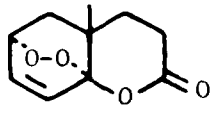
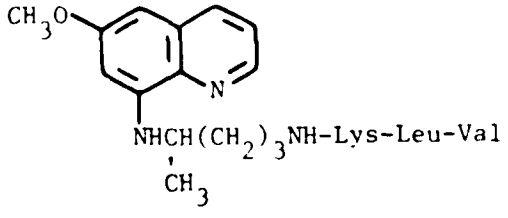
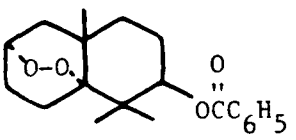
Identification[a]	Structure	Dose mg/kg	T-C[b]	Toxic Deaths[c]	MST[d]	Comments
RTI-2597-11 BK73145		40 160 640	-0.1 0.1 0.3	0 0 0	0 0 0	
RTI-2597-12 BK73798		40 160 640	-0.1 -0.1 -0.1	0 0 0	0 0 0	
RTI-2597-13 BK74482		40 160 640	0.0 0.0 0.2	0 0 0	0 0 0	
RTI-2597-15 BK94788		2.5 5.0 10.0 20.0 40.0 160 640	0.4 0.0 0.4 0.4 0.4 - -	0 0 0 0 0 5 5	0 0 0 0 0 0 0	 Toxic Toxic
RTI-2597-16 BK94797		2.5 5.0 10.0 20.0 40.0 160 640	0.4 0.6 1.4 2.0 3.2 - -	0 0 0 0 0 5 5	0 0 0 0 0 3 3	 Toxic Toxic

Table 2 (continued)

Identification[a]	Structure	Dose mg/kg	T-C[b]	Toxic Deaths[c]	MST[d]	Comments
RTI-2597-17		40	0	0	0	
BK95909		160	0	0	0	
		640	0	2	4	Toxic

a) The top number is the Research Triangle Institute identification. The bottom number is the Walter Reed Army Institute of Research identification.

b) The mean survival time of the treated group minus the mean survival time of the control group.

c) Deaths occurring on days 2, 3, 4 and 5 after infection are attributed to drug action and counted as toxic deaths. Control animals do not die before day 6.

d) The mean survival time for the mice dying on days 3, 4 and 5 only (toxic deaths).

terminal side chain amino function was not active but toxic at 40, 160 and 640 mg/kg.

3.3 Presumptive Causal Prophylactic Screen

These tests were carried out by the Rane Laboratory, University of Miami, Miami, Florida, using sporozoite-induced P. berghei yoelii infected mice.[22-25] The test compound was dissolved or suspended in 0.5% hydroxyethylcellulose-0.1% Tween·80 and administered either orally (po) or subcutaneously (sc) at several dose levels to groups of five mice on the day of challenge. Prophylactic activity is evidenced by survival of drug treated mice to 30 days. Survival of 40% or more of the mice in the treated group may be considered as indication of activity. Deaths of negative control mice occur between day 7 and day 15. Mortality occurring before day 7 may, therefore, be ascribed to the toxicity of the test compound.

Test results for ten (10) compounds is shown in Table 3. Compound RTI-2497-13, which showed 2/5 cures at 160 mg/kg, would be considered active at this dose. All other compounds were inactive at all three dose levels tested.

3.4 Radical Curative Test against P. Cynomolgi in Rhesus Monkeys

In this test, Indian rhesus monkeys (2-4 kg) are infected by IV injection of 10^6 freshly isolated P. cynomolgi sporozoites on day 0. A rapidly rising parasitemia develops after a 7-9 day prepatent period, and administration of the test drug is initiated when the rising parasite count exceeds 5000 per mm^3 (typically day 10-12). Test drugs are normally administered orally (by nasogastric intubation) once daily for 7 consecutive days in aqueous solution or, if insoluble, in suspension in 0.3% methylcellulose solution. Chloroquine diphosphate is always administered concurrently with the test drug for 7 days to eliminate blood schizonts. Thus, any tissue schizonticidal activity of the test drug will always be apparent even if it lacks blood schizonticidal

Table 3. Causal Prophylactic Activity against Plasmodium berghei yoelii

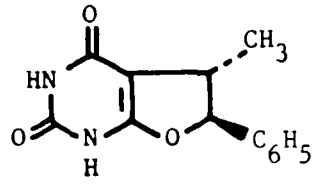
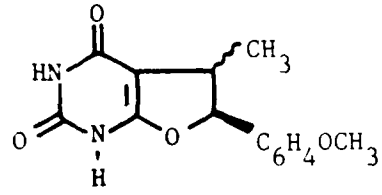
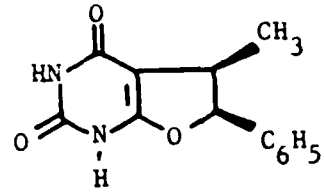
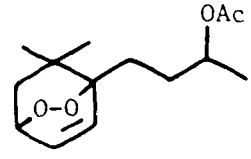
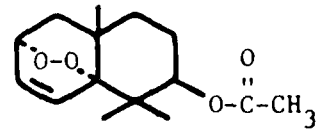
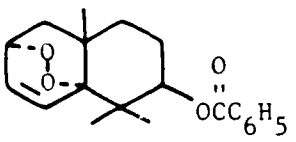
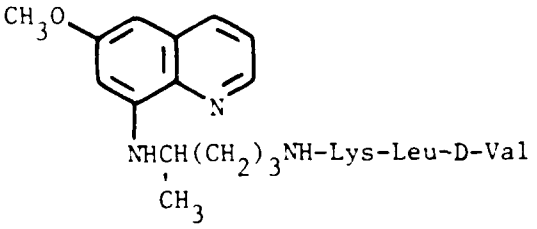
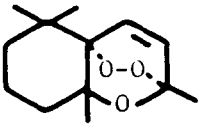
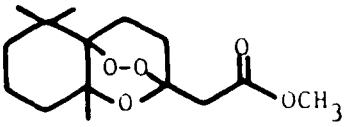
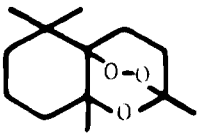
Identification[a]	Structure	Dose mg/kg	Cures SC	Cures PO	Toxic
RTI-2597-1 BK64995		10 40 160	0/5 0/5 0/5		
RTI-2597-2 BK69758		10 40 160	0/5 0/5 0/5		
RTI-2597-3 BK64986		10 40 160	0/5 0/5 0/5		
RTI-2597-4 BK69641		10 40 160	0/5 0/5 0/5		
RTI-2597-5 BK69892		10 40 160	0/5 0/5 0/5		

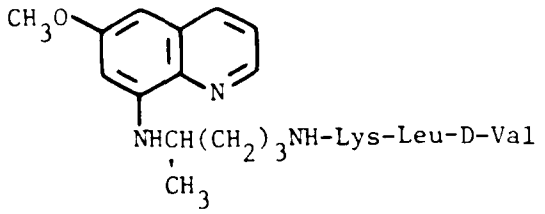
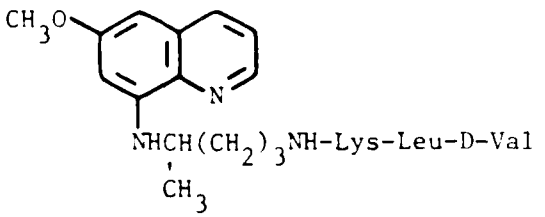
Table 3 (continued)

Identification[a]	Structure	Dose mg/kg	Cures SC	Cures PO	Toxic
RTI-2597-6 BK69909		10 40 160	1/5 0/5 0/5		
RTI-2597-7 BK70484		10 40 160	0/5 0/5 0/5		1 (sc)
RTI-2597-11 BK73145		10 40 160	0/5 0/5 0/5		
RTI-2597-12 BK73798		10 40 160	0/5 0/5 0/5		1 (sc) 1 (sc)
RTI-2597-13 BK74482		10 40 160	0/5 0/5 2/5		

activity. A vehicle control monkey and a positive drug control (primaquine) monkey are included in each group of inoculated monkeys. The effect of the test drug is determined by counting blood parasites. Parasite counts are made daily through day 20, and every 2 days thereafter. Initially a clearance of blood parasites is observed due to the blood schizonticidal action of chloroquine. If exoerythrocytic parasites ("tissue schizonts") survive the action of the test drug (i.e., if the drug is inactive or incompletely active), there will be a "relapse" of blood parasites. If there is no relapse within 20 days of the initial clearance of parasitemia, the monkey is splenectomized and its parasitemia followed for an additional 30 days. If there is no relapse within this period, the experiment is terminated, and the monkey is considered "cured." Primaquine diphosphate cures 90% of monkeys in this test system when administered at a dose of 1.3 mg/kg per day for 7 days in combination with chloroquine.

Two compounds were tested for radical curative activity against Plasmodium cynomolgi in rhesus monkeys at the SEATO Medical Research Laboratory, Bangkok.[26,27] The results are listed in Table 4. Note that the dose in the parenthesis is the amount of primaquine in the test pro-drug. At doses of 0.1 mg/kg (0.032 mg/kg of primaquine) and 1.0 mg/kg (0.319 mg/kg of primaquine) of RTI-2497-7, monkeys relapsed on day 6 through 9. When two monkeys were dosed at 1.0 mg/kg (0.319 mg/kg of primaquine) with the pro-drug RTI-2597-7, the monkeys relapsed on day 79 and day 65. Two monkeys have not relapsed after 30 and 31 days when dosed at 3.16 mg/kg (1.0 mg/kg of primaquine). Pro-drug RTI-2497-14 did not cure one monkey at a dose of 1.0 mg/kg (0.319 mg/kg of primaquine). Two monkeys have not relapsed after 58 and 49 days when dosed at 3.16 mg/kg (1.0 mg/kg) of primaquine.

Table 4. Antimalarial Activities of Primaquine Pro-drugs against P. Cynomolgi in Rhesus Monkeys

Identification[a]	Structure	Dose[b] mg/kg	Results
RTI-2597-7[c] BK70484		0.1 (0.032) 0.1 (0.032)	Relapse post Rx day 6 Relapse post Rx day 7
		0.316 (0.10) 0.316 (0.10)	Relapse post Rx day 6 Relapse post Rx day 9
		1.0 (0.319) 1.0 (0.319)	Relapse post RX day 79 Relapse post Rx day 65
		3.16 (1.0)	Negative for parasitemia at day 30
		3.16 (1.0)	Negative for parasitemia at day 21
RTI-2597-14[d] BK74491		1.0 (0.319)	Relapse post Rx day 13
		3.16 (1.0)	Negative for parasitemia at day 58
		3.16 (1.0)	Negative for parasitemia at day 49

a) The top number is the Research Triangle Institute identification. The bottom number is the Walter Reed Army Institute of Research identification.

b) The value in the parenthesis is the mg/kg of primaquine in the dose of the test compound. This was calculated from the following: MW of primaquine ÷ MW of test compound x dose (mg/kg) = mg/kg of primaquine in test compound.

c) Tested as diphosphate hydrate.

d) Tested as diphosphate 2.5 hydrate.

4.0 Experimental

Melting points were determined on a Koffler hot stage. Infrared (IR) spectra were recorded on a Perkin-Elmer 457 spectrophotometer. Ultraviolet spectra were run on a Varian model 2290 spectrophotometer. Proton magnetic resonance (^{1}H NMR) spectra were obtained on a Bruker 250 spectrometer. Chemical shifts were reported in δ values relative to tetramethylsilane (Me_4Si). Carbon magnetic resonance (^{13}C NMR) spectra were determined at 22.4 MHz on a JEOL FX-90Q spectrometer. Chemical shifts are reported in parts per million (ppm), and the δ scale referenced to the DMSO-d_6 solvent using 39.5 ppm relative to Me_4Si.

Potassium Azodicarboxylate (PADA).[9] Azodicarboxamide (Aldrich Chemical Co., 250 g) was added to 1.5 L of cold potassium hydroxide solution (40% w/v) in small portions over a period of 1.5 h. The temperature was kept below 8°C during addition. After being stirred an additional 5 h, the bright yellow dipotassium azodicarboxylate was separated by filtration, and the solid was washed 20 times with a total of 1 gallon of methanol precooled to 0°C. The solid was thoroughly dried under reduced pressure and stored in a desiccator over $CaSO_4$ in the refrigerator.

2-Benzoyloxy-1,1,10-trimethyl-6,9-epidioxydecalin (6a). To a flask containing a stirred mixture of 50 mL of CH_2Cl_2 and 50 mL of MeOH cooled in an ice-NaCl bath (∼ -10°C) was added 5a[4] (2.3 g, 0.007 mol) and potassium azodicarboxylate (27.3 g, 0.14 mol). Acetic acid (8.4 g) in 50 mL of CH_2Cl_2 was added dropwise over a period of 45 min and stirred overnight while allowing the temperature to rise to 23-25°C. The solid residue was removed and washed with CH_2Cl_2. The filtrate and washings were combined and evaporated to dryness to give 2.31 g of product. Recrystallization from CH_2Cl_2-pentane gave 1.94 g (84%) of 6a: mp 146-148°C; ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 3, 1-CH_3), 1.18

(s, 3, 1-CH_3), 1.29 (s, 3, 10-CH_3), 4.12 (m, 1, HC-O-O-), 5.14 (m, 1, H-C-O) and 7.39-8.03 (m, 5, aromatics).

Anal. Calcd for $C_{20}H_{26}O_4$: C, 72.70; H, 7.93. Found: C, 72.80; H, 7.97.

2-Butyloxycarbonyloxy-1,1,10-trimethyl-6,9-epidioxydecalin (6b). The reduction of the olefinic analog 5b[4] was carried out as described for the benzoyloxy analog 5a. Thus, to a stirred suspension of 5b (3.24 g, 0.010 mol) and potassium azodicarboxylate (39.1 g, 0.2 mol) in 120 mL of a mixture of 50% CH_2Cl_2 and MeOH at -5° to 0°C was added acetic acid (12.0 g, 0.2 mol) in 50 mL of CH_2Cl_2 over a period of 3.5 h. The stirred reaction mixture was allowed to warm to 23-25°C and remained overnight. Enough water was added to the mixture to dissolve all of the solids. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (50 mL). The combined extracts were washed with NaCl solution and dried ($MgSO_4$). Evaporation of the solvent gave 3.16 g (97%) of a waxy substance which solidified under vacuum. Recrystallization from hexanes gave 2.60 g (80%) of the target compound 6b: mp 46-48°C; ^1H NMR ($CDCl_3$) δ 0.93 (t, 3, CH_2CH_3), 1.03 (s, 6, C_1-CH_3), 1.23 (s, 3, C_{10}-CH_3), 4.11 (m, 3, -OCH_2 and C_6H), 4.74 (dd, 1, C_2H).

Anal. Calcd for $C_{18}H_{30}O_5$: C, 66.23; H, 9.26. Found: C, 66.34; H, 9.27.

2-Acetoxy-1,1,10-trimethyl-6,9-epidioxydecalin (6c). The reaction was carried out as described for 6a. Thus, 2.95 g (0.011 mol) of 5c was reduced with 42.0 g (0.22 mol) of potassium azodicarboxylate and 13.2 g (0.22 mol) acetic acid. After work-up, the residue was recrystallized from CH_2Cl_2-hexanes to give 2.21 g (74%) of 6c: mp 131-133°C; ^1H NMR ($CDCl_3$) δ 0.97 (s, 3, C_1-CH_3), 1.02 (s, 3, C_1-CH_3), 1.24 (s, 3, C_{10}-CH_3), 2.02 (s, 3, $COCH_3$), 4.1 (m, 1, HCOO-), 4.89 [dd, 1, HC-OC(O)CH_3].

Anal. Calcd for $C_{15}H_{24}O_4$: C, 67.23; H, 9.04. Found: C, 67.14; H, 9.06.

2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin (6d). Compound 6d was prepared as described for 6a-c. Thus, 5d (2.8 g, 0.0125 mol) with potassium azodicarboxylate (46.8 g, 0.24 mol) and acetic acid (14.4 g, 0.24 mol) gave after recrystallization from CH_2Cl_2-pentane 2.51 g (87%) of 6d: mp 148-149°C; ^1H NMR ($CDCl_3$) δ 0.95 (s, 3, C_1-CH_3), 1.08 (s, 3, C_1-CH_3), 1.22 (s, 3, C_{10}-CH_3), 3.68 (dd, 1, >C<u>H</u>OH), 4.08-4.18 (m, 1, H-C-OO-).

Anal. Calcd for $C_{13}H_{22}O_3$: C, 67.13; H, 9.02. Found: C, 67.14; H, 9.06.

2,4a-Epidioxy-3,4,5,6,7,8-hexahydro-2,5,5,8a-tetramethyl-2H-1-benzopyran (10). The endoperoxide 9[4] (3.1 g, 0.014 mol) in 150 mL of methanol was cooled in an ice-bath. Under nitrogen and with vigorous stirring potassium azodicarboxylate (PADA) (35 g, 0.17 mol) was added in one portion. To the yellow suspension was added dropwise a solution of methanol and acetic acid (30 mL, 1:1) over a period of 2 h. Vigorous gas evolution was noted. The bright yellow color of the suspension gradually faded to a white suspension. The precipitate was separated by filtration, and the filtrate and washings were combined and concentrated to give 2.6 g of yellow solid. Recrystallization from cold methanol gave 2.0 g of 10 as a white solid: mp 55-57°C; IR ($CHCl_3$) 3000-2800 (CH), 1385 (geminal dimethyl) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.94 (s, 3, C_5-CH_3), 0.96 (s, 3, C_5-CH_3), 1.25 (s, 3, C_{8a}-CH_3) and 1.38 (s, 3, C_2-CH_3).

Anal. Calcd for $C_{13}H_{22}O_3$: C, 68.99; H, 9.80. Found: C, 69.08; H, 9.81.

β-(1-Methyl-2-oxocyclohexyl)propionitrile (13a).[10] Acrylonitrile (106.4 g, 2.01 mol) was added dropwise to a stirred solution of 2-methylcyclohexanone (900 g, 8.02 mol) and "Triton B" (benzyltrimethylammonium hydroxide 40% in methanol, 1.0 g for each 40 g of cyclohexanone). The reaction temperature was maintained at 30-35°C by external cooling. The mixture was stirred at room temperature overnight and neutralized with 10% (v/v) HCl solution. The product was extracted with ethyl acetate twice, dried (sodium sulfate), filtered and

concentrated. The desired product was distilled under reduced pressure to provide 308.4 g of the nitrile 13a: bp [lit.[10] bp 132°C (1 mm)]; IR ($CHCl_3$) 2215 (C≡N), 1720 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 60 MHz) δ 1.15 (s, 3, C_4-CH_3).

β-(1-Methyl-2-oxocyclohexyl)propionic Acid (14a).[10] The nitrile 13a (75 g, 0.47 mol) was refluxed with 800 mL of aqueous sodium hydroxide (10% w/v) for 24 h. The reaction mixture was cooled in an ice bath and extracted three times with ether. The aqueous phase was acidified with concentrated HCl solution to pH 1. The acidic aqueous phase was extracted with $CHCl_3$. The organic extracts were combined, dried (sodium sulfate), filtered, concentrated and distilled to give 64.5 g of keto acid 14a as an oil: mp (lit.[10] mp 48°C); IR ($CHCl_3$), 3500-3100 (COOH), 1710 (C=O) cm^{-1}.

4a-Methyl-3,4,4a,5,6,7-hexahydro-2H-1-benzopyran (15a).[11] A solution of keto acid 14a (172.5 g, 0.94 mol) in freshly distilled acetic anhydride (1.5 L) and acetyl chloride (500 g) was heated to reflux for 48 h. Excess reagents were distilled under reduced pressure. The residue was dissolved in ether and washed with dilute sodium bicarbonate solution (5% w/v), dried (sodium sulfate), filtered and concentrated to give 160 g of dark brown oil. Purification by column chromatography (SiO_2, CH_2Cl_2-hexanes 1:1) provided 90 g of the desired enol-lactone 15a as an oil: IR ($CHCl_3$) 1758 (C=O), 1680 (C=C) cm^{-1}; ^1H NMR (CDC13) δ 1.15 (s, 3, C4-CH3), 2.7 (bt, 2, J = 8 Hz, C2H) and 5.20 (m, 1, C_8H).

4a-Methyl-3,4,4a,5-tetrahydro-2H-1-benzopyran (16a). The enol-lactone 5a (30 g, 0.18 mol) in 750 mL of dry carbon tetrachloride was heated to gentle reflux. Recrystallized N-bromosuccinimide (33 g, 0.18 mol) was added all at once. The resulting suspension was irradiated with a 150 W light for 15 min. The precipitate was separated by filtration, and the filtrate and carbon tetrachloride washings were combined and treated immediately with 35 mL of

N,N-dimethylaniline. The mixture was heated briefly to reflux and quenched in crushed ice. The product was extracted with $CHCl_3$, and the chloroform extract was washed several times with 10% HCl (v/v) solution, dried (sodium sulfate), filtered and concentrated to give a brown oil, which was purified by SiO_2 column chromatography (CH_2Cl_2) to afford 9.5 g of the diene-lactone 16a: IR ($CHCl_3$), 1768 (C=O), 1659, 1590 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.18 (s, 3, C_{4a}-CH_3), 5.6 (d, 1, J = 5.5 Hz, C_7H), 5.62 (m, 1, C_7H) and 5.8 (m, 1, C_6H).

This product was unstable and was used in the next reaction without further purification.

6,8a-Epidioxy-4a-methyl-2-oxo-3,4,4a,5,6,8a-hexahydro-2H-1-benzopyran (17a). The diene-lactone 16a (5.0 g, 0.030 mol) in 100 mL of methylene chloride was irradiated with a GE quartzline BWY-650 lamp in the presence of oxygen and polymer bonded rose bengal sensitizer (2.0 g). The reaction was complete after 3 h as indicated by TLC analysis. The rose bengal dye was removed by filtration and washed with CH_2Cl_2. The filtrate and washings were combined and concentrated to give a solid, which was placed in 1:1 Et_2O-Hexanes and sonicated to a fine powder, then decanted. This procedure was repeated 3 times to give 2.8 g (48%) of 17a as off-white crystals: mp 139-141°C; IR ($CHCl_3$) 1765 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.05 (s, 3, C_4-CH_3), 4.67 (m, 1, C_6H), 6.54 (d, 1, J = 10 Hz, C_8H) and 6.66 (dd, 1, J = 5, 10 Hz, C_7H).

Anal. Calcd for $C_{10}H_{12}O_4$: C, 61.22; H, 6.17. Found: C, 61.31; H, 6.19.

β-(1,5-Dimethyl-2-oxo-cyclohexyl)propionitrile (13b). A 500 mL three necked flask equipped with a condenser and addition funnel was charged with 75 mL (0.596 mol) of 2,4-dimethylcyclohexanone then flushed with N_2. The reaction was placed in a water bath (22°C) and 2.1 g (0.019 mol) of potassium t-butoxide was added. The resulting mixture quickly became thick, as 13.9 mL (0.211 mol) of acrylonitrile was added dropwise. The reaction mixture thinned

out considerably with the addition. The yellow solution was stirred overnight, then acidified with 3 N HCl. This suspension was poured into 200 mL of ether and extracted with brine. The organic layer was dried ($MgSO_4$), filtered and concentrated. Vacuum distillation of the concentrate gave 21.3 g (57%) of pure 13b: bp 116-120°C (0.20 mm); R_f 0.44 (10% Et_2O-hexanes); IR (neat) 2980, 2940, 2880, 2257, 1710, 1460, 1430, 1385, 1130, 1110, 1045 and 1010 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.98 (d, 3, J = 6 Hz), 1.01 (s, 3, C_2-CH_3), 1.32 (t, 2, J = 13 Hz), 1.80 (m, 2), 2.05 (m, 3), 2.28 (m, 3), and 2.51 (dt, 1, J = 6, 13 Hz, C_4H); mass spectrum m/z (relative abundance) 179 (M^+, 4), 134 (100), 126 (62), 120 (30) and 95 (58).

Anal. Calcd for $C_{10}H_{17}NO$: C, 73.69; H, 9.55; N, 7.82. Found: C, 73.51; H, 9.56, N, 7.80.

β-(1,5-Dimethyl-2-oxo-cyclohexyl)propionic Acid (14b). A heterogeneous solution of β-(1,5-dimethyl-2-oxocyclohexyl)propionitrile (13b, 12.0 g, 0.0670 mol) and 150 mL of 10% (w/w) aqueous sodium hydroxide was refluxed for 15 h. The turbid yellow mixture turned a clear yellow. The reaction was cooled and acidified to pH 0 with 6 N HCl. The cloudy suspension was extracted 2 times with methylene chloride (150 mL) and dried (Na_2SO_4). The solution was filtered and concentrated in vacuo to afford a 13.1 g (98%) of chromatographically pure acid 14b: R_f 0.40 (5% acetone-methylene chloride); IR (neat) 3400-2900 (-OH), 2960, 2930, 2880, 1740, 1710, 1460, 1420, 1380, 1200, 1130 and 740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.95 (d, 3, J = 6 Hz, C_5-CH_3), 0.98 (s, 3), 1.28 (m, 2, C_1-CH_3), 1.78 (m, 2), 2.16 (m, 6), and 2.56 (dt, 1, J = 2,6 Hz, C_5H); mass spectrum m/z (relative abundance) 198 (M^+, 27), 154 (100), 139 (17), 126 (82), 108 (21), 96 (33), 94 (28), 55 (47), 43 (21), 41 (62).

Anal. Calcd for $C_{11}H_{18}O_3$: C, 66.62; H, 9.14. Found: C, 66.53; H, 9.18.

4a,6-Dimethyl-2-oxo-3,4,4a,5,6,7-hexahydro-2H-1-benzopyran (15b). A solution of 5.0 g (0.025 mol) of keto-acid 14b in 75 mL of acetic anhydride was cooled to 0°C. Acetyl chloride (25 mL) was added dropwise over a 0.5 h period. After completion of addition, the mixture was refluxed for 48 h. The volatiles were removed via distillation under vacuum (25 mm). The residue was dissolved in Et_2O and extracted successively with water, saturated sodium bicarbonate and brine solution. The organics were dried (Na_2SO_4), filtered and concentrated in vacuo to afford 3.16 g (70%) of crystalline 15b: mp 37-39°C; R_f 0.77 (5% acetone-CH_2Cl_2); 0.49 (1:1 Et_2O-hexanes); IR (neat) 2960, 2920, 2859, 1745, 1675, 1455, 1340, 1280, 1210, 1160, 1120, 1035, 920, 820 and 640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.93 (d, 3, J = 4 Hz, C_6-CH_3), 1.12 (s, 3, C_{4a}-CH_3), 1.52 (t, 2, J = 6 Hz, C_4H), 1.78 (m, 3), 2.21 (s, 2, C_1H), 2.61 (t, 2, J = 6 Hz, C_3H), and 5.27 (t, 1, J = 3 Hz, C_8H).

Anal. Calcd for $C_{11}H_{16}O_2$: C, 73.28; H, 8.94. Found: C, 73.41; H, 8.90.

4a,6-Dimethyl-2-oxo-3,4,5,6-tetrahydro-2H-1-benzopyran (16b). To a 250 mL round bottom flask was added 5.25 g (0.029 mol) of 15b and 70 mL of predried (Na_2SO_4) CCl_4. A condenser fitted with a drying tube was put into place and the solution was heated to reflux. At this point, 5.49 g (0.031 mol) of N-bromosuccinimide was added and the resulting suspension irradiated with a 300 W bulb (at a distance of greater than 10 cm). After an induction period of approximately 30 s, the suspension foamed vigorously. The reaction was stirred for 15 min, whereupon irradiation was ceased. The mixture was filtered through celite after cooling. To the clear yellow filtrate was added 3.56 mL (0.031 mol) of N,N-dimethylaniline. This homogenous solution was heated at reflux for 2 h with condenser and drying tube in place. A red-green oil separated over this time. The mixture was cooled and concentrated in vacuo to a thick oil. The residue was dissolved in 4 mL of CH_2Cl_2, then

diluted with 100 mL of pentane, which causes the clear solution to become cloudy. After separation of the reddish oil was complete the pentane was decanted and retained. This procedure was repeated 3-5 times. The combined pentane layers were washed twice with 0.05 N HCl, then brine, and dried (sodium sulfate). At this point, the diene is one spot by TLC. The solution was filtered and concentrated to yield 4.71 g (91%) of pure diene 16b: R_f 0.37 (20% EtOAc-hexanes); IR (neat) 2980, 2920, 2880, 1710, 1660, 1610, 1450, 1380, 1330, 1275, 1250, 1160, 1020, 820 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.19 (s, 3, C_{4a}-CH_3), 1.76 (s, 3, C_6-CH_3), 1.85 (broad m, 4), 2.76 (t, 2, J = 6 Hz, C_3H), 5.41 (s, 2, C_7H, C_8H).

This product was used in the next step without further purification.

4a,6-Dimethyl-6,8a-epidioxy-2-oxo-3,4,4a,5,6,8a-hexahydro-2H-1-benzopyran (17b). A solution of 4.81 g (0.027 mol) of diene 16b in 80 mL of dry THF was placed in a water jacketed, externally irradiated (300 W) oxygenation apparatus. To this was added approximately 50 mg of hematoporphyrin as sensitizer. The solution was irradiated for 6 h at 23°C with periodic monitoring by TLC for disappearance of starting material. The reddish mixture was flushed through a short column of silica gel, then chromatographed (20% EtOAc-hexanes) to yield 2.44 g (43%) of pure endoperoxide 17b as a waxy solid: IR (neat) 2975, 2940, 1765, 1660, 1450, 1370, 1245, 1185, 1140, 1050, 1025, 1000, 930, 845, 740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.06 (s, 3, C_{4a}-CH_3), 1.39 (s, 3, C_6-CH_3), 1.73 (d of q, 2, J = 2.5, 7 Hz, C_5H), 2.56 (m, 2), 2.72 (m, 2), 6 40 (d, 1, J = 9 Hz, C_8H), 6.51 (d, 1, J = 9 Hz, C_7H); mass spectrum M^+ - 32 calculated for $C_{11}H_{14}O_2$ m/z 178.0994, observed m/z 178.0993.

Anal. Calcd for $C_{11}H_{14}O_4$: C, 62.84; H, 6.71. Found: C, 62.85; H, 6.72.

6,8a-Epidioxy-4a-methyl-2-oxo-3,4,4a,5,6,7,8,8a-octahydro-2H-1-benzopyran (18a). A three necked 250 mL flask was charged with 112 mg (0.571 mmol)

of epidioxide olefin 17a and was dissolved in 5 mL of methylene chloride under N_2. The solution was cooled to -20°C and potassium azodicarboxylate (PADA, 1.35 g, 0.0086 mol) added in one portion. A solution of 230 μL of glacial acetic acid in 1.5 mL of methylene chloride was added dropwise over 1 h. The addition was repeated 4 times at 4 h intervals for a total addition of 920 μL of acetic acid. The resultant yellow suspension was allowed to stir at -20°C for 12 h (24 h total). Filtration through celite was followed by concentration in vacuo to afford an off-white solid. This was taken up in 1:1 ether-methylene chloride and gravity filtered. The filtrate was concentrated, yielding a yellow solid. ^{1}H NMR analysis showed it to be a 96:4 mixture of 18a and 17a with a yield of 83 mg (73%): R_f = 0.61 (10% EtOAc-Hexanes); IR (KBr) 2940, 175, 1450, 1260, 1165, 110, 1075, 960, 900, 885, 780 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.25 (s, 3, C_{4a}-CH_3), 1.50-2.40 (bm, 8), 2.59 (s, 2) and 4.18 (bs, 1, C_6H),

This reaction was repeated on a 1.6 g scale to give 512 mg (32%) of 18a, mp 74-78°C.

Anal. Calcd for $C_{10}H_{14}O_4$: C, 60.53; H, 7.17. Found: C, 60.41; H, 6.99.

10-Methyl-2-oxo-1(9),7-hexalin (29b).[14] Enone 27b[4] (10.0 g, 0.060 mol) was dissolved in 800 mL of warm, anhydrous t-butanol. Chloranil (80 g, 0.32 mol) was added, and the suspension was heated at reflux for 3 h. The mixture was cooled, filtered and concentrated. The residue was taken up in ether and washed with water, 5% NaOH, water, then brine. The organic layer was dried (magnesium sulfate), filtered, and concentrated to a green oil. Distillation afforded 3.2 g (32%) of 29b as a yellow oil: bp 82-85°C (0.15 mm) [lit.[14] bp 70-71°C (0.05 mm)]; IR 2920, 1660, 1620, 1590; ^{1}H NMR ($CDCl_3$) δ 1.20 (s, 3, C_{10}-CH_3), 1.71 (m, 4), 2.46 (m, 4), 5.60 (s, 1, C_1H), 6.17 (bs, 2, C_7H, C_8H).

7,10-Dimethyl-2-oxo-Δ^8-octalin (30b).[14] A dry 250 mL 2-necked flask was fitted with a septum and a T-joint. One arm of the T-joint was connected to a

vacuum pump, the other to a nitrogen line. Copper cyanide (1.845 g, 0.020 mol) was added, the flask was evacuated, and then filled with dry nitrogen. Dry toluene (6 mL) was added, and the flask was carefully evacuated until all of the toluene had distilled off. This procedure was repeated 2 times with a total of 12 mL of toluene. THF (60 mL) was then added, and the flask was cooled to -78°C. Methyl lithium (24.6 mL, 1.56 M, 38.42 mmol) was added, and the well stirred solution was warmed to 0°C for 10 min, at which time no solid was visible in the flask, and the solution was clear. The flask was cooled to -30°C, and a solution of 3.00 g (2.72 mL, 18.75 mmol) of dienone 29b in 10 mL of dry THF was added dropwise. The reaction was complete in 1 h (GC) and quenched with saturated NH_4Cl. The cloudy, green suspension was diluted with ether and poured into dilute ammonium chloride, extracted and washed twice with water. The organics were dried (sodium sulfate), filtered and concentrated in vacuo to a thick yellow clear oil. This was chromatographed on silica gel (10% EtOAc-Hexanes) to give 2.81 g (86%) of the unsaturated ketone 30b as an unstable oil: IR (neat) no OH, 2920, 2860, 1710, 1450, 1240; ^1H NMR ($CDCl_3$) δ 0.92 (d, 3, J = 7 Hz, C_7-CH_3), 1.22 (s, 3, C_{10}-CH_3), 5.20 (t, 1, J = 3 Hz, C_8H).

Since compound 30b decomposed on standing, it was used directly in the next step.

7,10-Dimethyl-2β-hydroxy-Δ^8-octalin (31b). A solution of 2.16 g (0.012 mol) of octalin 30b in 30 mL of dry THF in a 100 mL flask under nitrogen was cooled to -78°C. Diisobutyl aluminum hydride (DIBAL, 1.0 M in hexanes, 13.6 mL, 13.6 mmol) was added dropwise, and the solution was stirred at -78°C while monitored by GC. After 1 h, the reaction was complete. The solution was quenched with saturated ammonium chloride, then poured into 5% Rochelles salt (potassium sodium tartrate), extracted with ether, and the ether washed

twice with water. The ether layer was dried (Na_2SO_4), filtered, concentrated in vacuo, and the resdiue chromatographed (SiO_2, 20% EtOAc-Hexanes). This gave 412 mg of the faster moving isomer and 1.62 g of 31b as the slower eluting compound. Overall yield was 2.03 g (94%) of a 20:80 ratio of the two alcohols.

Compound 31b: R_f = 0.44 (20% EtOAc-Hexanes); IR 3340, 2920, 2860, 1460, 1240, 1050 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.95 (d, 3, J = 7 Hz, C_7-CH_3), 1.02 (s, 3, C_{4a}-CH_3), 1.95-1.10 (bm, 9), 2.18 (d, 2, J = 8 Hz, C_1H), 3.45 (m, 1, C_2H) and 5.23 (s, 1, C_8H).

Isomer of 31b: R_f = 0.54 (20% EtOAc-Hexanes); ^1H NMR ($CDCl_3$) δ 0.91 (d, 3, J = 7 Hz), 1.05 (s, 3), 2.20-1.20 (bm, 11), 3.99 (bs, 1), and 5.28 (s, 1).

7,10-Dimethyl-2β-acetoxy-Δ^8-octalin (32b). A 10 mL flask containing 501 mg (2.77 mmol) of homoallylic alcohol 31b, 459 μL (4.16 mmol) of acetic anhydride and 578 μL (4.16) mmol) of triethylamine was placed under nitrogen. To this well stirred solution was added a catalytic amount of dimethylaminopyridine (DMAP, 24 mg), and the mixture was allowed to stir for 12 h, at which time the reaction was complete by TLC. The solution was poured into ether, washed twice with 1 N HCl once with saturated $NaHCO_3$ and was dried (sodium sulfate). Filtration followed by concentration and chromatography (SiO_2, 10% EtOAc-Hexanes) gave a quantitative (614 mg) yield of the corresponding acetate 32b: R_f = 0.50 (10% EtOAc-hexanes); IR 2920, 1730, 1430, 1360, 1230, 1020 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.91 (d, 3, J = 8 Hz, C_7-CH_3), 1.05 (s, 3, C_{4a}-CH_3), 1.90-1.20 (bm, 9), 1.98 (s, 3, $OCOCH_3$), 2.19 (d, 2, J = 8 Hz, C_1H), 4.51 (m, 1, C_2H), and 5.22 (d, 1, J = 2Hz, C_8H).

2-Oxo-1(9),7-hexalin (29a).[18] A three necked 1 L flask was fitted with a dry-ice condensor, addition funnel and stopper. Ammonia (300 mL) was condensed into the flask and kept at or below -45°C. 2-Methoxynaphthalene (7.0 g,

0.044 mol) was suspended in 50 mL of warm ethanol and quickly added to the ammonia. Metallic sodium (7.0 g, 0.304 g-atom) was added in small lots, and a dark blue color developed. Upon addition of all the sodium, the suspension was slowly warmed to room temperature. The blue color dissipated quickly, and the ammonia was allowed to evaporate. The residue was diluted with EtOAc, and water was carefully added. The water layer was separated, and the organics were washed twice with water. The EtOAc extracts were concentrated to a thick oil. The oil was dissolved in 100 mL of 5% sulfuric acid, and the solution was heated to reflux for 2 h. Upon cooling, the mixture was poured into EtOAc, and the acid layer removed. The EtOAc layer was washed twice with water and once with saturated $NaHCO_3$. The organics were dried (sodium sulfate), filtered, concentrated and distilled at high vacuum to give 4.2 g (65%) of a pale yellow, light sensitive oil: bp 114-117°C (1.5 mm) [lit.[18] bp 137-140°C (12 mm); R_f = 0.13 (10% EtOAc-Hexanes); IR 3040, 2940, 2880, 2840, 1660, 1620, 1585, 1325, 1260, 1205, 870, 750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30-2.80 (m, 9), 5.70 (s, 1, C_1H), and 6.22 (s, 2, C_7H, C_8H). The oil is unstable and was normally used within 24 h.

10-Methyl-2-oxo-Δ^8-octalin (30a).[14] A 2 necked 25 mL flask was fitted with a septum and a T-joint. Copper cyanide (127 mg, 1.42 mmol) was dried as reported above. Dry THF (5.0 mL) was added, and the well stirred suspension was cooled to -78°C. Methyl lithium (1.75 mL, 1.56 M (hexane), 2.73 mmol) was added dropwise, and the increasingly clear solution was warmed to 0°C for 10 min, at which time solid was no longer visible in the flask. This solution was recooled to -30°C, and the dienone 29a (181 μL, 1.22 mmol) was added neat. After 1 h, an aliquot of the orange reaction mixture showed no evidence of starting material by GC. The reaction was quenched with saturated ammonium chloride. The resulting green-yellow suspension was poured into ether and was

washed three times with water. The organics were dried (Na_2SO_4), filtered and concentrated to a clear oil. The oil was chromatographed on silica gel (10% EtOAc-Hexanes) to afford 182 mg (85%) of the unsaturated ketone 30a: R_f = 0.54 (10% EtOAc-Hexanes); IR 2930, 287, 1710, 1600, 1450, 1260, 1175, 965, 750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.97 (d, 3, J = 7 Hz, C_7-CH_3), 2.39 (t, 2, J = 4 Hz, C_3H), 1.20-2.60 (bm, 8), 2.98 (s, 2, C_1H), and 5.28 (s, 1, C_8H).

10-Methyl-2β-hydroxy-Δ^8-octalin (31a). A solution of 3.82 g (0.024 mol) of 30a in 60 mL of dry THF, under nitrogen, was cooled to -78°C. Diisobutyl aluminum hydride [DIBAL, 1.0 M (hexane), 28 mL, 28 mmol] was added dropwise, and the mixture was stirred for 1 h at -78°C. The reaction was quenched with saturated ammonium chloride, and poured into 5% Rochelles salt. The ether layer was isolated, washed twice with water, then dried (sodium sulfate). Filtration and concentration in vacuo were followed by chromatography (silica gel, 10% EtOAc-Hexanes) to give 643 mg of the more mobile isomer and 1.63 g of the slower moving isomer which combined with 1.51 g of mixed fractions gave a 98% yield.

Compound 31a: R_f = 0.14 (10% EtOAc-Hexanes); IR 3430, 2920, 2860, 1450, 1355, 1050, 840 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.95 (d, 3, J = 7Hz, C_7-CH_3), 1.20-2.20 (bm, 10), 3.35 (bm, 1, C_2H), and 5.27 (bs, 1, C_8H).

Faster moving isomer: R_f = 0.29 (EtOAc-Hexanes); IR 3480, 2920, 2860, 1650, 1450, 1370, 1070, 1000, 840, 830 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.97 (d, 3, J = 7 Hz), 1.20-2.20 (bs, 10), 2.21 (s, 2), 4.00 (bs, 1) and 5.32 (s, 1).

10-Methyl-7β-acetoxy-Δ^8-octalin (32a). A 10 mL flask containing 150 mg (0.903 mmol) of homoallylic alcohol 31a was placed under nitrogen. Acetic anhydride (137 μL, 1.35 mmol) was added, followed by 173 μL (1.35 mmol) of triethylamine, then 8 mg dimethylaminopyridine (DMAP), and the mixture was stirred for 12 h, at which time the reaction was complete by TLC. The mixture

was poured into ether, washed twice with 1 N HCl, once with water, once with saturated sodium bicarbonate, and dried (sodium sulfate). Filtration, concentration and chromatography (silica gel, 10% EtOAc-Hexanes) gave a quantitative (187 mg) yield of the corresponding acetate 32a: R_f = 0.53 (10% EtOAc-Hexanes); IR 2920, 2860, 1725, 1450, 1380, 1230, 1030, 850 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.92 (d, 3, J = 7 Hz, C_7-CH_3), 1.92 (s, 3, $OCOCH_3$), 2.00-1.20 (bm, 10), 2.35 (dd, 2, J = 4, 12 Hz, C_1H), 4.51 (bm, 1, C_2H), 5.22 (s, 1, C_8H).

Carbobenzyloxy-D-alanylleucine Methyl Ester. Starting with leucine methyl ester hydrochloride (7.28 g, 0.040 mol) and Cbz-D-alanine (8.92 g, 0.04 mol), the peptide ester was prepared following the same procedure for BOC-valylleucine methyl ester.[4] The ester was purified by crystallization from ether-pentane mixture to yield 10.87 g (78%) of product: mp 69-70°C; ^1H NMR ($CDCl_3$) δ 0.91 [d, 6, $-C(CH_3)_2$], 1.38 (d, 3, $CHCH_3$), 3.71 (s, 3, OCH_3), 5.11 (s, 3, $-CH_2-O$), 7.283 (s, 5, aromatic).

Carbobenzyloxy-D-alanylleucine (44b). A solution of Cbz-D-alanylleucine methyl ester prepared in the previous experiment (1.16 g, 0.0033 mol) in 3 mL of DMF and 18 mL of acetone was stirred with 4.5 mL of 1N NaOH at room temperature for 1 h. The solvents were removed under reduced pressure, and the residue was dissolved in 60 mL of H_2O and extracted with ether. The aqueous layer was acidified with solid citric acid and extracted with ethyl acetate. The ethyl acetate solution was washed with NaCl solution, dried (Na_2SO_4) and evaporated to yield 1.05 g (94%) of 44b as an oil: ^1H NMR ($CDCl_3$) δ 0.92 [d, 6, $-C(CH_3)_2$], 1.38 (d, 3, $-CH_3$), 7.29 (s, 5, aromatics).

The free acid (1.0 g, 0.003 mol) was dissolved in ethyl alcohol, mixed with dicyclohexylamine (0.55 g, 0.003 mol) and diluted with ether to give a white crystalline precipitate. Filtration gave the salt which was washed with ether and dried under vacuum to give 1.12 g (71%) of 44b dicyclohexylammonium salt: mp 153-156°C.

The hydrolysis of the ester was repeated on a larger scale with approximately the same yield, and the oily free acid was used in subsequent reactions.

4'-N[Cbz-D-Ala-Leu-(N^{ε}-Boc-Lys)]primaquine (45b). To a stirred solution of 43 (0.88 g, 0.0018 mol), 44b (0.71 g, 0.0018 mol), and HOBT (0.26 g, 0.0018 mol) in 3 mL of DMF at 0° was added dropwise a solution of dicyclohexylcarbodiimide (0.42 g, 0.002 mol) in 5 mL of methylene chloride. After 4.5 h the reaction mixture was diluted with 25 mL of ethyl acetate. The resulting precipitate was separated by filtration. The residue obtained on evaporation of the filtrate was purified by column chromatography (silica-gel 60 g). The column was eluted with 10% ethyl acetate in methylene chloride to give 0.94 g (64%) of 45b. Recrystallization from ethyl acetate-hexanes gave 0.63 g of 45b as pale yellow crystals: mp 55-56°C; ^{1}H NMR ($CDCl_3$) δ 0.91 [d, 6, $-C(CH_3)_2$], 1.24 (d, 3, $-CH_3$), 1.42 [s, 9, $-C(CH_3)_3$], 3.87 (s, 3, $-OCH_3$), 5.01 (s, 2, $-CH_2-O$), 7.25 (s, 5, phenyl).

This experiment was repeated twice on 0.01 and 0.005 mole scale to give 60 and 63% yields.

4'-N-(D-Ala-Leu-Lys)primaquine (46b). A solution of 45b (7.44 g, 0.095 mol) in 100 mL of 50% trifluoroacetic acid in CH_2Cl_2 was stirred at room temperature for 1 h. The deep red solution was evaporated under reduced pressure at 20-25°C and dried under vacuum. The residue was triturated with ether and the ether solution decanted. The solid residue was dissolved in 150 mL of MeOH and hydrogenated in the presence of Pd/C (0.75 g) for 2 h. The catalyst was separated by filtration, and the residue after evaporation of the solvent was chromatographed on silica gel. Elution with $CHCl_3$-MeOH-NH_4OH (80:18:4) gave 3.75 g (71%) of 46b. This was dissolved in 10 ml of MeOH and treated with 1.5 g of 85% H_3PO_4 to give 3.54 g of a pale cream colored solid.

The solid was recrystallized from H_2O-EtOH to give 3.32 g of 46b phosphate salt: mp 123-128°C (dec); ^1H NMR (D_2O) δ 0.86 (d, 6, isopropyl), 1.28 (d, 3, $CHCH_3$), 3.87 (s, 3, OCH_3), 6.41, 7.41, 7.96 and 8.49 aromatics.

Anal. Calcd for $C_{43}H_{63}N_7O_8 \cdot 2H_3PO_4 \cdot 2.5H_2O$: C, 44.33; H, 7.44; N, 12.07. Found: 44.15; H, 7.15; N, 12.12.

Cbz-Valylleucine (44d). Cbz-Valylleucyl methyl ester (6.00 g) was dissolved in 75 mL of acetone and 12.5 mL of DMF. Sodium hydroxide (18 mL of 1 N) was added, and the mixture was stirred at room temperature for 1 h. Most of the solvents were removed under reduced pressure. The residue was dissolved in 250 mL of H_2O and extracted with ether. The aqueous layer was acidified to pH 4 with solid citric acid, and the free acid was extracted with ethyl acetate. The ethyl acetate solution was washed with saturated NaCl solution and dried over Na_2SO_4.

Evaporation of the solvent under reduced pressure gave 5.4 g of 44d as a thick viscous semisolid. This material was used in the next step without further purification.

4'-N-[Cbz-L-Val-Leu(N^{ε}-Boc-Lys)]primaquine (45d). The intermediate 45d was prepared as described for 45b. Thus, N^{ε}-Boc-Lys-primaquine (7.27 g, 0.015 mol) was condensed with Cbz-Val-Leu-OH (44d) (5.4 g, 0.15 mol) to give 9.97 g (81%) of 45d after purification by column chromatography: ^1H NMR ($CDCl_3$) δ 1.35 (s, 9, t-Bu), 3.82 (s, 3, OCH_3), 5.04 (s, 2, $PhCH_2$), 7.35 (s, 5, phenyl).

4'-N-(Val-Leu-Lys)primaquine (46d). Removal of the Cbz and Boc groups was accomplished as described for 46b. Thus, 9.5 g (0.012 mol) of 45d was hydrogenated in the presence of 900 mg of Pd/C in methanol. After removal of the catalyst and the solvent, the residue was treated with 50 mL of trifluoroacetic acid in 50 mL of CH_2Cl_2. The resulting product was purified by column chromatography to give 5.10 g (71%) of 46d.

A solution of the free base in 15 mL of MeOH was treated with 0.98 g of 85% H_3PO_4. The solution was diluted with absolute ethanol to induce crystallization. The cooled mixture was filtered to give 3.85 g of 46d. The sample was recrystallized from H_2O-EtOH to yield 2.93 g of pure 46d as the diphosphate salt: mp 194-196°C; ^1H NMR (D_2O) δ 0.81 [d, 6, $CH(C\underline{H}_3)_2$], 1.00 [d, 6, valyl $CH(C\underline{H}_3)_2$], 1.28 (d, 3, $CHC\underline{H}_3$), 3.91 (s, 3, OCH_3), 6.47-6.62, 7.48, 8.12 and 8.53 (aromatics).

Anal. Calcd for $C_{32}H_{35}N_7O_4 \cdot 2H_3PO_4 \cdot \frac{1}{2}H_2O$: C, 47.75; H, 7.51; N, 12.18. Found: C, 47.91; H, 7.52; N, 11.86.

5.0 References

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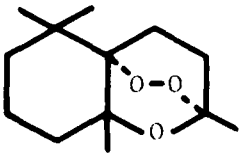
6.0 Appendix

Table of Compounds Submitted to WRAIR

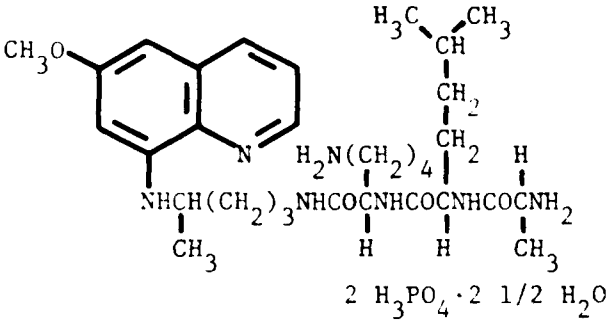
1. 2,4a-Epidioxy-3,4,5,6,7,8-hexahydro-2,5,5,8a-tetramethyl-2H-1-benzopyran



BK74482
RTI-2597-13
10 from this report
target compound
2.0 g shipped

Synthesis is described on page 39.

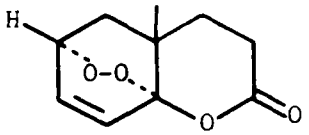
2. 4'-N-(D-Ala-Leu-Lys)primaquine Diphosphate



BK74491
RTI-2597-14
46b from this report
target compound
3.2 g shipped

Synthesis is described on page 51.

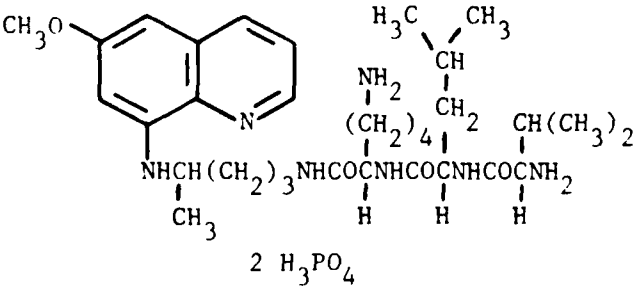
3. 6,8a-Epidioxy-4a-methyl-2-oxo-3,4,4a,5,6,8a-hexahydro-2H-1-benzopyran



BK94788
RTI-2597-15
17a from this report
target compound
2.01 g shipped

Synthesis is described on page 41.

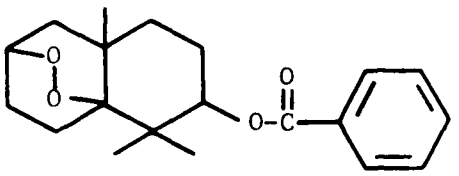
4. 4'-N-(Val-Leu-Lys)primaquine Phosphate



BK94797
RTI-2597-16
46d from this report
target compound
2.86 g shipped

Synthesis is described on page 52.

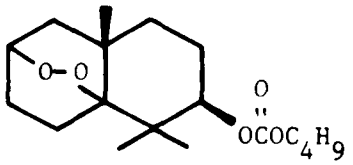
5. 2-Benzoyloxy-1,1,10-trimethyl-6,9-epidioxydecalin



BK95909
RTI-2597-17
6a from this report
target compound
2.06 g shipped

Synthesis is described on page 37.

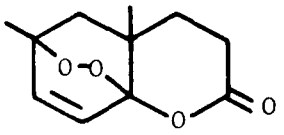
6. 2-Butyloxycarbonyl-1,1,10-trimethyl-6,9-epidioxydecalin



BK97029
RTI-2597-18
6b from this report
target compound
2.5 g shipped

Synthesis is described on page 38.

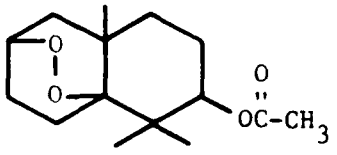
7. 4a,6-Dimethyl-6,8a-epidioxy-2-oxo-3,4,4a,5,6,8a-hexahydro-2H-1-benzopyran



BK97038
RTI-2597-19
17b from this report
target compound
1.81 g shipped

Synthesis is described on page 44.

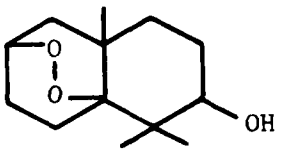
8. 2-Acetoxy-1,1,10-trimethyl-6,9-epidioxydecalin



BL03764
RTI-2597-20
6c from this report
target compound
2.01 g shipped

Synthesis is described on page 38.

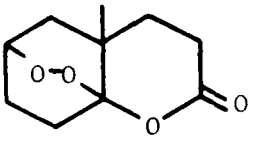
9. 2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin



BL04243
RTI-2597-21
6d from this report
target compound
2.01 g shipped

Synthesis is described on page 39.

10. 6,8a-Epidioxy-4a-methyl-2-oxo-3,4,4a,5,6,7,8,8a-octahydro-2H-1-benzopyran



BL07146
RTI-2597-22
18a from this report
target compound
0.635 g shipped

Synthesis is described on page 44.

7.0 Distribution

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